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A review of the performance of rapid diagnostic tests for the NS1 protein in diagnosing dengue infection

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Abstract

Introduction: The dengue virus is composed of structural proteins (envelope, membrane, and capsid) and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The NS1 protein is the most relevant, as its hexameric form is released into the blood and can be detected in this medium, in serum and plasma. Rapid diagnostic tests are presented as an alternative to detecting dengue infection through this protein due to their accessible format.

Objectives: To establish a review of the performance of three commercial rapid diagnostic tests available in Mexico, based on the detection of the NS1 protein for the diagnosis of dengue through sensitivity and specificity values.

Methods: Twelve retrospective diagnostic research studies were reviewed from scientific databases that included the SD Bioline Dengue Duo ABBOTT, CERTUM Rapid Dengue Combo Test Cassette, and Standard F Dengue NS1 Ag FIA SD Biosensor tests and reported their sensitivity and specificity values.

Conclusions: Most of the articles reviewed show good performance, with the highest being the Standard F Dengue NS1 Ag FIA SD Biosensor. However, factors such as primary and secondary infections, serotypes, and cross-reactions also play an important role in the performance of these tests.

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Keywords: Dengue virus; Rapid diagnostic test; NS1 protein; Lateral flow assay; Infectious disease.

Introduction

The dengue virus is classified as an arbovirus with four distinct serotypes: DENV 1-4 throughout tropical and subtropical regions [1-3]. Rapid Diagnostic Tests (RDT) are tools for improving the diagnosis of dengue infections in remote or low-resource areas, can be used with basic training and results available in less than 20 minutes. However, there are conflicting reports regarding the sensitivity and specificity of these tests [4,5]. This review aims to provide an overview of current international and national reports on the sensitivity and specificity of three rapid tests for the NS1 protein that are commercially available and easily accessible.

Materials and methods

This review considered retrospective diagnostic accuracy research articles published in English and Spanish. The articles were retrieved from scientific databases such as PubMed, Research Gate, Web of Science and Google Scholar between March 2024 and May 2025, as described in Figure 1. Additionally, articles published within the last five years were given priority. Among the inclusion criteria, the use of at least one of the following tests had to be reported in the literature consulted: SD Bioline Dengue Duo ABBOTT (11FK46), CERTUM Rapid Dengue Combo Test Cassette (IDEC-425), and Standard F Dengue NS1 Ag FIA SD Biosensor (10DEN10D), which examine the NS1

antigen and, with the exception of the latter test, also assess IgM and IgG. The above rapid tests were considered because they are commercially available and easily accessible in Mexico.

The following search terms were used to search for information:

1. RDT dengue
2. SD Dengue Duo
3. Rapid Diagnostic Test dengue
4. SD Bioline Dengue Duo
5. Standard F NS1 Ag
6. (RDT)(dengue)

The evaluated literature had to include at least one of the following aspects in the study population: a history of dengue infection, location in an endemic area for the vector, or presentation of symptoms characteristic of the infection, such as high fever. In addition, a healthy control group or a group with another type of infection had to be included in the studies. Another criterion for the inclusion of articles was that a reference standard had to be present for at least each of the antigens and antibodies evaluated. In addition, it was necessary for them to include the sensitivity and specificity values for the NS1 protein. The values for IgM/IgG could be included or not included.

Those articles without a validated reference standard or sensitivity and specificity values for NS1 were excluded. No restrictions were placed on the age of the study population, the biological material used (serum, plasma, or blood), the identification of primary or secondary infection, or the onset of symptoms for each item.

Results

The identification of thirty retrospective diagnostic accuracy research articles was the result of the initial research. Priority was given to the most recent articles published within the last five years, thus excluding seventeen articles published between 2010 and 2019. One more article was discarded because it did not report the NS1 specificity value. After applying the inclusion and exclusion criteria, only twelve articles were included in this review The most widely used rapid test during the review of the articles was the SD Bioline Dengue Duo ABBOTT, which accounted for 7 (58.3%) of the total number of studies. The Standard F Dengue NS1 Ag FIA SD Biosensor was the second most popular, with four (33.3%) articles, and finally, the CERTUM Rapid Dengue Test Combo Cassette had one article (8.3%). It should be mentioned that, to date, the cited study is the only one in which the performance of this last test has been reported.

A total of 12 articles were developed in endemic regions, except for the work of Matusali *et al.* [6] carried out their work in a non-endemic area (Italy) but with samples from people with a history of international travel. All studies described inclusion criteria regarding age ranges for the febrile population with a minimum age of >9 months and no maximum age. Gender was not considered an exclusion criterion for any article, as the sample population was selected based on febrile samples suspected of dengue infection. For this same reason, researchers conducted epidemiological surveillance studies or test performance studies in the study area during past epidemic outbreaks. As for the control groups, samples from individuals with fever due to other viral or bacterial infections were included in eleven articles, and just one article also included blood donors and healthy patients, further favoring the evaluation of specificity. The biological material most used for both groups was serum (8), followed by plasma (3) and finally blood (2). The most widely used reference standard for identifying the dengue virus and its serotypes was RT-PCR. All the articles presented report its use for comparison with the NS1 protein, although other techniques were also used, such as in the study by Yow *et al.* [7], which used viral isolation and an immunofluorescence test to identify the serotype. For serological tests, four articles used ELISA for IgM as the reference standard, while three articles used IgG alone. Another technique used for serological testing was the plaque reduction neutralization technique, also considered a reference standard, as described by Zammarchi *et al.* [8].

The SD Bioline Dengue Duo Abbott rapid diagnostic test and the CERTUM Rapid Dengue Test Combo Cassette identify the NS1 protein, as well as IgM and IgG. In contrast, the Standard F Dengue NS1 Ag FIA SD Biosensor detects only NS1. The performance of the tests for NS1 alone was ranked in a sensitivity range of 38.6%–90% and a specificity range of 87.7%–100%. Between each test, the best sensitivity performance of SD Bioline Dengue Duo was 90% compared to the lowest, which was 38.6%. In terms of specificity, the range was between 90% and 100%, while the sensitivity range of Standard F Dengue NS1 Ag FIA was 79.11%–100% and its specificity was 87.5%–100%. Overall, the Standard F Dengue NS1 Ag FIA SD Biosensor performed best in terms of sensitivity for NS1. It also had the best specificity values, along with the SD Bioline Dengue Duo ABBOTT.

A total of four articles evaluated primary infection with a reference standard method for IgM and the reported performance range was 4.4%–83.3% for the SD Bioline Dengue Duo ABBOTT. The reports showed conflicting results when evaluated with NS1, with some indicating benefits and others showing no clear advantages. Liu *et al.* [9] report that adding IgM or IgG increases the rapid test's sensitivity in identifying secondary infections. This finding is supported by Yow *et al.* [7] and Chong *et al.* [10], who also found better results when IgM was included with NS1 than when NS1 was used alone. However, for the work of Kikuti *et al.* [11], the addition of IgM to the NS1 assessment is not sufficient for an increase in sensitivity values.

Regarding the evaluation of secondary IgG infection, sensitivity was reported in only two articles, with the best performance being that of Santoso et al. [12] at 60%. However, the result may be influenced by the large difference in the number of samples that were analyzed.

In general, the serotypes of the dengue virus are evaluated in all articles, except for Zapata *et al.* [13] and Zammarchi *et al.* [8]. All articles identify the four serotypes except Kikuti *et al.* [11], who only described DENV-1, DENV-3, and DENV-4.

Additionally, five articles evaluated cross-reactivity with other flaviviruses, viruses from other families, and bacteria. Two articles evaluated these characteristics with SD Bioline Dengue Duo ABBOTT test. The study by Santoso *et al.* [12] reports that this test does not cross-react with any of the evaluated infections, achieving 100% specificity. In contrast, the study by Yow *et al.* [7] it was evaluated against samples of early and convalescent Zika infection, as well as samples of Chikungunya, and the test did not react with any of them. Two other articles evaluated the cross-reaction of the Standard F Dengue NS1 Ag FIA SD Biosensor. First, in the study by Zammarchi *et al.* [8], of the

total number of samples negative for dengue, cross-reaction occurred for the Toscana virus, rheumatoid factor, and Epstein-Barr virus, with only one case reported for each condition. Second, Zuroidah *et al.* [14] study reports 100% specificity, indicating that there was no cross-reaction with other infections. Information on the overall performance of rapid diagnostic tests is summarized in Table 1.

Table 1: Performance of three rapid diagnostic tests for the NS1 protein of the dengue virus commercially available in Mexico.

Test	Author	Sample size	Patient characteristics	Sample type	Referenced method	Sensitivity	Specificity
SD BIOLINE dengue duo	Kikuti M *et al.* [11]	Dengue cases: 246 Controls: 254	Suspected dengue, >5 y/o, 2-3 onset of feverControls, >5 y/o, blood donors, >50 kg	Serum	ELISA -NS1 ELISA-IgM RT-PCR	NS1: 38.6% IgM: 13.8% NS1/IgM: 46.8%	NS1: 98% IgM: 94.9% NS1/IgM: 88-96%
	Santoso et al. [12]	Dengue cases: 100 Controls: 49	Suspected dengue, <4 days and >4 days onset of fever Controls with febrile illnesses (malaria, typhoid fever and bacterial infections)	Serum	Panbio Dengue Duo IgM/IgG RT-PCR	NS1: 73 % IgM: 78.6% IgM/IgG: 60 %	NS1: 100%
	Liu *et al.* [9]	Dengue cases: 13 Controls: 37	Suspected dengue, >8 y/o, 0-6 onset of fever	Serum	RT-PCR	NS1: 89.7% NS1 o IgM: 95.6% NS1/IgM/IgG: 97.1%	NS1: 91.9% NS1 o IgM: 89.2% NS1/IgM/IgG: 86.5%
	Prabowo *et al.* [15]	Dengue cases: 99 Controls: 102	Suspected dengue, 1-60 y/o, <5 days and >6 days onset of fever	Serum	RT-PCR	NS1: 87.88%	NS1: 90%
	Yow *et al.* [7]	Dengue cases: 108 Controls: 30	Suspected dengue, 4-11 days onset of fever	Serum	RT-PCR ELISA NS1 ELISA IgM/IgG Viral isolation	NS1: 68.5% IgM: 83.3% NS1/IgM: 97.2%	NS1: 100%
	Chong *et al.* [10]	Dengue cases: 227 Controls: 262	Suspected dengue, <5 days and >6 days onset of fever	Capillary and venous blood	RT-PCR Capture IgM/IgG ELISA Panbio ELISA primary infection	NS1: 52.4% IgM: 45.4% IgG: 27.8% NS1 o IgM: 75.8%	NS1: 97.7% IgM: 98.5% IgG: 88.9% NS1 o IgM: 96.6% NS1, IgM o IgG: 87.4%
	Vickers *et al.* [16]	Dengue cases: 309 Controls: 30	Suspected dengue	Serum	ELISA antigen NS1 ELISA IgM/IgG	NS1: 90% IgM: 49.3% IgG: 39.1% NS1/IgM/IgG: 98.9%	NS1: 99.2% IgM: 95.9% IgG: 100% NS1/IgM/IgG: 100%
Casete combo prueba rápida	Zapata *et al.* [13]	Dengue cases: 45 Controls: 79	Suspected dengue, 2-7 days onset of fever				
Standard F*	Zammarchi *et al.* [8]	Dengue cases: 21 Controls: 36	Suspected dengue, <8 days onset of fever Controls with febrile illnesses (Zika, West Nile virus, Toscana virus, Epstein-Barr virus)	Serum	ELISA-DENV NS1 Ag RT-PCR ELISA IgM/IgG PRNT	NS1: 90%	NS1: 87.5%
	Zuroidah *et al.* [14]	Dengue cases: 50 Controls: 30	Suspected dengue,3-7 days onset of fever Controls with febrile illnesses (typhoid fever, malaria, hepatitis B, hepatitis C, and leptospirosis)	Serum	ELISA-DENV NS1 Ag RT-PCR	NS1: 82%	NS1: 100%
	Ruchusatsawat *et al.* [17]	Dengue cases: 158 Controls: 246	Suspected dengue, 2-4 days onset of fever Controls with febrile illnesses (Chikv, bacterial infections, others)	Plasma	RT-PCR ELISA IgM and ELISA IgG	NS1: 79.11%	NS1: 92.28%
	Matusali *et al.* [6]	Dengue cases: 65	Suspected dengue with history of international travel to endemic areas	Plasma Serum Blood	Real time RT-PCR	NS1: 84.6%	NS1: 100%

Discussion

Although the performance of rapid tests depends largely on the test itself, the result may be influenced by other factors such as sample collection time, viremia, serotype, and even antibodies responding to infection.

The best performance reported among the tests corresponds to Standard F, and it is important to note that although all three tests are lateral flow tests, detection in the aforementioned test is performed using fluorochromes or fluorescence-labeled particles that are detected by signal-sensitive equipment, unlike the other two tests, which use colloidal gold particles, among others, and show the test lines with the naked eye [18]. This difference could be significant, as the results are subject to the interpretation of each evaluator and biases could be introduced, as has been reported in other studies where a decision is reached by up to three evaluators [10].

The timing of sample collection also influences the results, as researchers have reported that during a primary infection, the NS1 protein remains in serum on average from the onset of symptoms until the sixth day, although some cases have also been found to remain until the ninth day [19,20]. Based on this reasoning, most of the articles reviewed sampled before the sixth day and obtained sensitivity values above 73%. Nevertheless, there are investigations such as that by Yow *et al*. [7] that reported reduced sensitivity values even though some of their samples are within the acceptable range. The beneficial effect on performance might be due to the specific proportion of samples collected before the sixth day. In the study mentioned, the proportion was 41/67 samples obtained before and after six days, respectively. As the majority are acquired after the seventh day, when the amount of NS1 in the blood starts to decrease [21], it is less probable that the examination will identify it, an outcome that is strengthened by the examination's high sensitivity to IgM, which rises after the fifth day [22].

On the other hand, Kikuti *et al.* [11] and Zapata *et al.* [13] reported lower sensitivity values despite collecting samples within the recommended range. Both studies were conducted in endemic areas constantly exposed to dengue infections, where it has been reported that in secondary infections, the NS1 protein decreases compared to a primary infection [23] due to an IgG response that reduces the viral load and, therefore, the concentration of soluble NS1. Also, it forms an NS1-IgG immune complex that blocks the epitopes of the NS1 protein, preventing them from being detected by the antibodies attached to diagnostic tests [24,25].

Serotypes also play a role in determining the sensitivity of rapid diagnostic tests. In their study, Liu et al. reports that the SD Dengue Duo test has higher sensitivity for detecting DENV-1 than DENV 2-4, a result has also been documented with other rapid tests, which report sensitivity values of 92.2% and 50% for DENV-1 and DENV-2, respectively [4,26]. This suggests that the sensitivity may have been affected by a wider distribution of DENV-2 samples.

Another factor that could also be related to the performance of rapid tests is cross-reactivity. However, in the studies evaluated, this result was minimal, as in the case of SD Bioline, which was compared in one study against cell cultures infected with variants of the Zika virus (Uganda, Thailand, and Puerto Rico) and concluded that under natural conditions these viruses would not express false positives since the viral titers are lower than those detected by rapid tests for NS1 of the Dengue virus [27]. Another type of rapid diagnostic test known as TKK2, evaluated in Bangladesh, has shown the same condition. This test did not cross-react with samples infected with the Zika virus [28], and it did not show any reaction when evaluated against RT-PCR [29]. A compilation of studies and their results in SD Bioline and Standard F indicate that rapid diagnostic tests based on the dengue NS1 protein are highly specific compared to other flaviviruses [30].

Conclusions

Rapid diagnostic tests facilitate the diagnosis of dengue infection and play a particularly important role in endemic areas where resources are limited, and even more so in certain emergency situations. Although the results were inconsistent in this review, most of the studies showed good results in detecting the NS1 protein and even against other infections. To ensure optimal performance, it is essential that the use of these rapid tests adhere to the guidelines provided by the suppliers, considering prior work, and the conditions of use. It is also important to mention that the constant evaluation of the performance of rapid diagnostic tests allows for the development of this technology to have a greater and more accurate ability to detect dengue infection.

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Appendix

Table 1: Performance of three rapid diagnostic tests for the NS1 protein of the dengue virus commercially available in Mexico.

Test	Autor	Sample size	Patient characteristics	Sample type	Referenced method	Sensitivity	Specificity
SD BIOLINE Dengue Duo	Kikuti M *et al.* [11]	Dengue cases: 246 Controls: 254	Suspected dengue, >5 y/o, 2-3 onset of fever Controls, >5 y/o, blood donors, >50 kg	Serum	ELISA -NS1 ELISA-IgM RT-PCR	NS1: 38.6% IgM: 13.8% NS1/IgM: 46.8%	NS1: 98% IgM: 94.9% NS1/IgM: 88-96%
	Santoso et al. [12]	Dengue cases: 100 Controls: 49	Suspected dengue, <4 days and >4 days onset of fever Controls with febrile illnesses (malaria, typhoid fever and bacterial infections)	Serum	Panbio Dengue Duo IgM/IgG RT-PCR	NS1: 73 % IgM: 78.6% IgM/IgG: 60 %	NS1: 100%
	Liu *et al.* [9]	Dengue cases: 13 Controls: 37	Suspected dengue, >8 y/o, 0-6 onset of fever	Serum	RT-PCR	NS1: 89.7% NS1 o IgM: 95.6% NS1/IgM/IgG: 97.1%	NS1: 91.9% NS1 o IgM: 89.2% NS1/IgM/IgG: 86.5%
	Prabowo *et al.* [15]	Dengue cases: 99 Controls: 102	Suspected dengue, 1-60 y/o, <5 days and >6 days onset of fever	Serum	RT-PCR	NS1: 87.88%	NS1: 90%
	Yow *et al.* [7]	Dengue cases: 108 Controls: 30	Suspected dengue, 4-11 days onset of fever	Serum	RT-PCR ELISA NS1 ELISA IgM/IgG Viral isolation	NS1: 68.5% IgM: 83.3% NS1/IgM: 97.2%	NS1: 100%
	Chong *et al.* [10]	Dengue cases: 227 Controls: 262	Suspected dengue, <5 days and >6 days onset of fever	Capillary and venous blood	RT-PCR Capture IgM/IgG ELISA Panbio ELISA primary infection	NS1: 52.4% IgM: 45.4% IgG: 27.8% NS1 o IgM: 75.8%	NS1: 97.7% IgM: 98.5% IgG: 88.9% NS1 o IgM: 96.6% NS1, IgM o IgG: 87.4%
	Vickers *et al.* [16]	Dengue cases: 309 Controls: 30	Suspected dengue	Serum	ELISA antigen NS1 ELISA IgM/IgG	NS1: 90% IgM: 49.3% IgG: 39.1% NS1/IgM/IgG: 98.9%	NS1: 99.2% IgM: 95.9% IgG: 100% NS1/IgM/IgG: 100%
Casete Combo Prueba Rápida	Zapata *et al.* [13]	Dengue cases: 45 Controls: 79	Suspected dengue, 2-7 days onset of fever				
Standard F*	Zammarchi *et al.* [8]	Dengue cases: 21 Controls: 36	Suspected dengue, <8 days onset of fever Controls with febrile illnesses (Zika, West Nile virus, Toscana virus, Epstein-Barr virus)	Serum	ELISA-DENV NS1 Ag RT-PCR ELISA IgM/IgG PRNT	NS1: 90%	NS1: 87.5%
	Zuroidah *et al.* [14]	Dengue cases: 50 Controls: 30	Suspected dengue,3-7 days onset of fever Controls with febrile illnesses (typhoid fever, malaria, hepatitis B, hepatitis C, and leptospirosis)	Serum	ELISA-DENV NS1 Ag RT-PCR	NS1: 82%	NS1: 100%
	Ruchusatsawat *et al.* [17]	Dengue cases: 158 Controls: 246	Suspected dengue, 2-4 days onset of fever Controls with febrile illnesses (Chikv, bacterial infections, others)	Plasma	RT-PCR ELISA IgM and ELISA IgG	NS1: 79.11%	NS1: 92.28%
	Matusali *et al.* [6]	Dengue cases: 65	Suspected dengue with history of international travel to endemic areas	Plasma Serum Blood	Real time RT-PCR	NS1: 84.6%	NS1: 100%

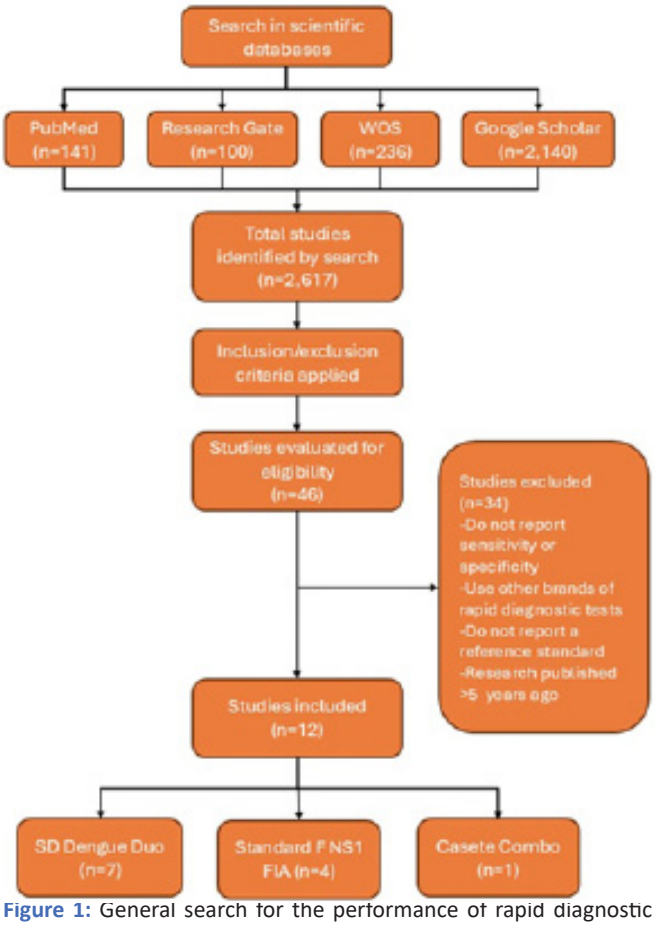

Figure 1: General search for the performance of rapid diagnostic tests for NS1.